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Single-nucleotide polymorphism panels in the racial authentication of Hairless pigs in Mexico

Paneles de polimorfismos de un solo nucleótido en la autenticación racial del cerdo Pelón en México

Painéis de polimorfismo de nucleotídeo único na autenticação racial de porco Pelado no México

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Rev. Fac. Agron. (LUZ). 2025, 42(3): e254231
ISSN 2477-9407
DOI: https://doi.org/10.47280/RevFacAgron(LUZ).v42.n3.II

Animal production

Associate editor: Dra. Rosa Razz
University of Zulia, Faculty of Agronomy
Bolivarian Republic of Venezuela

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Received: 01-04-2025
Accepted: 02-06-2025
Published: 30-06-2025

Keywords:
SNP panel
Animal genetic resources
Native pigs

Abstract

Massive genotyping panels of single-nucleotide polymorphisms (SNPs) were evaluated to create an authentication and racial identification strategy for the Hairless pig. Three populations of Hairless pigs from the states of Nayarit (n=10), Oaxaca (n=10) and Yucatán (n=143), Mexico, were genotyped with the porcine-GGP-50K chip, and genotypes for the Duroc (n=66), Hampshire (n=33), Landrace (n=95), Large White (n=47), Pietrain (n=42) and Iberico hairless (n=15) breeds were added. Three strategies involving previously reported SNP panels and a fourth strategy involving the combination of all SNP panels was evaluated. Using canonical discriminant analysis (CDA), the canonical correlations and percentages of racial discrimination were obtained, and with the first two canonical variables, distance trees between populations were constructed. Racial separation was achieved with all four strategies; the greater the number of SNPs used, the better the identification of the Hairless pig. The combined panel with 96 SNPs achieved 100 % racial assignment and had the greatest canonical correlation in the CDA, revealing a racial grouping of the three Hairless pig populations close to the Iberian population. With SNP panels, it is possible to achieve the racial authentication of the Hairless pig and discriminate it from other pig breeds.

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Resumen

Con el objetivo de contar con una estrategia de autentificación e identificación racial del cerdo Hairless, fueron valorados paneles de polimorfismos de nucleótido único (SNP) de genotipificación masiva. Tres poblaciones de cerdo Pelón de los estados de Nayarit (10), Oaxaca (10) y Yucatán (143) México, fueron genotipificados con el chip porcine-GGP-50K, asimismo, se adicionaron genotipos para las razas Duroc (66), Hampshire (33), Landrace (95), Large White (47), Pietrain (42) e Ibérico lampiño (15). Se valoraron tres estrategias de paneles reportados y una cuarta estrategia con la combinación de todos los paneles de SNP. Con el análisis de discriminación canónica (ADC) se obtuvieron los valores de correlación canónica de discriminación, porcentaje de discriminación racial y con las dos primeras funciones canónicas se realizaron los árboles de distancias entre poblaciones. Las cuatro estrategias logran la separación racial, a mayor número de SNP empleados mejora la identificación del cerdo Pelón. El panel combinado con 96 SNP tuvo una asignación racial al 100 %, con mayor correlación canónica en el ADC, observándose un agrupamiento racial de las tres poblaciones de cerdo Pelón, cercano a Ibérico. Con los paneles de SNP se puede lograr la autentificación racial del cerdo Pelón y discriminarse de otras razas porcinas.

Palabras clave: panel SNP, cerdos nativos, recursos genéticos animales.

Resumo

Painéis massivos de genotipagem de polimorfismos de nucleotídeo único (SNPs) foram avaliados para criar uma estratégia de autenticação e identificação racial para porco Pelado. Três populações de porco Pelado dos estados de Nayarit (n=10), Oaxaca (n=10) e Yucatán (n=143), México, foram genotipadas com o chip porcine-GGP-50K, e genótipos para as raças Duroc (n=66), Hampshire (n=33), Landrace (n=95), Large White (n=47), Pietrain (n=42) e Iberico hairless (n=15) foram adicionados. Três estratégias envolvendo painéis de SNP relatados anteriormente e uma quarta estratégia envolvendo a combinação de todos os painéis de SNP foram avaliadas. Usando análise discriminante canônica (CDA), as correlações canônicas e porcentagens de discriminação racial foram obtidas e, com as duas primeiras variáveis canônicas, árvores de distância entre populações foram construídas. A separação racial foi alcançada com todas as quatro estratégias; quanto maior o número de SNPs utilizados, melhor a identificação do porco Pelado. O painel combinado com 96 SNPs alcançou 100 % de atribuição racial e teve a maior correlação canônica no CDA, revelando um agrupamento racial das três populações de porco Pelado próximo à população ibérica. Com os painéis de SNP, é possível alcançar a autenticação racial do porco Pelado e discriminá-lo de outras raças de suínos.

Palabras-chave: painel SNP, recursos genéticos animais, suínos nativos.

Introduction

The use of single-nucleotide polymorphisms (SNPs) in the genotyping of pig breeds enables the performance of population studies and the identification of genes associated with production. Another important use of SNP genotyping is racial discrimination and the authentication of individuals in each breed (Muñoz *et al*., 2020). SNP panels identified with 60K chips from Illumina (www.illumina.com) and 50K chips from Neogen (www.neogen.com) have been used in racial discrimination (Wilkinson *et al*., 2012; Schiavo *et al*., 2020; Moretti *et al*., 2022; Pasupa *et al*., 2020). Various statistical methodologies have been used to obtain these discriminant SNP panels, such as average Euclidean distance (AED), fixation index (FST), principal component analysis (PCA), partial least squares regression (PLSR), canonical discriminant analysis (CDA) and differences in allelic frequencies (Miao *et al*., 2023). A reference states that the Hairless pig arrived in Mexico through the Caribbean Islands on the second voyage of Christopher Columbus in the year 1493; since that date, the Hairless pig has been distributed from the Yucatan Peninsula to the shores of the Gulf of Mexico in the center, southwest and west regions of Mexico, and its genetic closeness with the Iberian pig has been recognized (Lemus-Flores *et al*., 2023). In Mexico, the Hairless pig is important in rural nutrition; however, interest in its production for use in gourmet tourism and the nutraceutical market has increased. According to the Food and Agriculture Organization (FAO, 2022), the Hairless pig is in danger of extinction; however, the FAO does not provide data on its characterization, so it is necessary to characterize and authenticate this breed to provide value, differentiate products and confirm its identity in conservation and rescue programs (Lemus-Flores *et al*., 2023). The objective of this study was to use a SNP panel strategy for racial discrimination of the Hairless pig to aid in the differentiation of animals that reproduce in local populations.

Materials and methods

Reference for animals and data collection

Three populations of Hairless pigs from the states of Nayarit (n=10), Oaxaca (n=10) and Yucatán (n=143), Mexico, were used to validate the SNP panels, and the populations were genotyped with the GGP Porcine 50K chip (GeneSeek Genomic Profiler Porcine) (Lemus-Flores *et al*., 2023). DNA extraction, purification and PCR process were performed by the Neogen company (www.neogen.com). The low number of Hairless pig animals in Nayarit and Oaxaca was due to the fact that they are small populations and a relationship sample was selected. Genotypes of the Duroc (n=66), Hampshire (n=33), Landrace (n=95), Large White (n=47), Pietrain (42) and Iberian hairless (n=15) breeds were added to the Illumina 60K chip database from previous studies (Yang *et al*., 2017). Individuals that were assigned to their racial group by all the discrimination strategies used were added to their breed in the database.

Racial verification strategy

Three separate SNP panels and a fourth strategy combining the SNPs from all three panels (Moretti *et al*., 2022; Schiavo *et al*., 2020; Wilkinson *et al*., 2012) were used in this study. The three separate panels included 12 SNPs (Moretti *et al*., 2022), 38 SNPs (Schiavo *et al*., 2020), and 49 SNPs (Wilkinson *et al*., 2012), and the fourth panel included the 96 SNPs from these three panels. Common SNPs between chips with the power of discrimination and racial allocation were identified.

Discrimination analysis

According to the methodology of Moretti *et al*. (2022), for each panel, the genotypes were assigned a value of 0 when the minor allele frequency (MAF) indicated that the genotype was homozygous for the major allele, 1 when the genotype was heterozygous, and 2 when the genotype was homozygous for the minor allele. Using SPSS v9, canonical discriminant analysis (CDA) was performed to create a predictive model of group membership in each panel. Using these four SNP panels and the combined databases including all races, the linear discriminant canonical variables that provide the best possible discrimination between the groups were identified, and the prediction equation adjusted for the racial identification and discrimination of Hairless pigs was obtained. Considering the canonical correlations of discrimination, percentage of racial discrimination, and distance trees between populations for the four panels, the best strategy to discriminate Hairless pigs from other breeds was determined.

Results and discussion

Panels

The SNP panels formed according to the SNPs identified by the two chips are presented in table 1. There are three SNPs in the 12-SNP panel that are repeated in the 49-SNP panel.

Assessment of racial discrimination

According to the canonical correlations of the first two variables in the CDA, the panels with the most SNPs, those with 49 and 96 SNPs, are the panels with the greatest racial discrimination among the four panels studied, as observed in table 2.

Based on the racial grouping according to canonical variables 1 and 2, the 96-SNP panel exhibited the best separation among the groups (figure 1).

Racial grouping

In the CDA for racial grouping, the only panel that did not correctly group 100 % of Hairless pigs was the 38-SNP panel; three Hairless pigs were predicted as Landrace pigs, and one was predicted as a Pietrain pig (Table 3).

The separation of the racial groups indicated that the Hairless pigs are close to the Iberian, Hampshire and Duroc pigs, as shown in figure 2.

In the separation of the Hairless pig populations with the 96 SNP panel, the populations of Nayarit, Oaxaca and Yucatán were grouped together (figure 3).SNP panels identified with high-density chips have been used in several species, and a high degree of racial assignment was achieved with fewer than 100 SNPs in bovines (Wilkinson *et al*., 2011). Another strategy for racial identification uses SNPs associated with candidate genes with productive characteristics that are highly expressed in certain races, such as the studies published by Óvilo *et al*. (2022) and Palma-Granados *et al*. (2023) in Iberian pigs and Zhang *et al*. (2014) in commercial pigs. Wang *et al*. (2022) constructed a reference panel of haplotypes to impute genotypes to different pig breeds with increased efficiency.

Table 1. SNPs in each panel (*Sus domesticus*).

Panel No.	SNP					
12	ALGA0027544	ALGA0047912	ASGA0004735	DBMA0000205	H3GA0016973	INRA0002279
	INRA0029816	INRA0052808	M1GA0006536	MARC0027620	MARC0038980	MARC0042228
38	ALGA0026433	ALGA0033636	ALGA0038216	ALGA0047010	ALGA0047511	ALGA0055609
	ALGA0060332	ALGA0061762	ALGA0062298	ALGA0088377	ALGA0092770	ALGA0093763
	ALGA0096892	ALGA0105829	ALGA0111963	ALGA0119129	ALGA0119566	ASGA0028870
	ASGA0054824	ASGA0066512	ASGA0103220	DRGA0008467	DRGA0011340	DRGA0016582
	H3GA0024339	H3GA0032382	H3GA0043731	H3GA0046254	H3GA0048198	H3GA0056051
	INRA0039368	INRA0039430	M1GA0007506	M1GA0021082	MARC0004055	MARC0019146
	MARC0038400	MARC0081387				
49	ALGA0001762	ALGA0003076	ALGA0003145	ALGA0010391	ALGA0010777	ALGA0026051
	ALGA0036101	ALGA0042134	ALGA0042589	ALGA0047912	ALGA0048114	ALGA0071850
	ALGA0074932	ALGA0085893	ALGA0103648	ALGA0106261	ALGA0108841	ASGA0014878
	ASGA0019578	ASGA0024792	ASGA0025238	ASGA0038785	ASGA0053943	ASGA0069860
	ASGA0073467	ASGA0073470	CASI0009493	DBMA0000259	DIAS0000043	DRGA0007655
	H3GA0013097	H3GA0021745	H3GA0045081	H3GA0053839	H3GA0056129	INRA0002279
	INRA0014142	INRA0022517	INRA0029891	INRA0029897	INRA0036473	INRA0040988
	MARC0029724	MARC0030810	MARC0038980	MARC0056888	MARC0061507	MARC0075425
	MARC0093043					

SNP: single-nucleotide polymorphism.

Table 2. Percentage of variance and canonical correlations in each SNP panel.

SNP	12		38		49		96	
Variable	% V	CC	% V	CC	% V	CC	% V	CC
1	50.0	.941	38.4	.931	56.9	.981	47.5	.988
2	28.1	.901	19.8	.877	16.8	.939	18.1	.969
3	14.6	.831	16.7	.859	10.4	.906	13.9	.960
4	4.9	.654	11.0	.806	8.2	.885	8.5	.937
5	1.9	.473	8.2	.761	5.3	.837	8.1	.934
6	.6	.293	5.9	.705	2.4	.719	3.9	.878

% V: percentage of variance. CC: canonical correlation. SNP: single-nucleotide polymorphism.

Table 3. Predicted membership group according to each SNP panel.

	Hairless	Duroc	Hampshire	Landrace	Large White	Pietrain	Iberian
SNP/n	163	66	33	95	47	42	15
12	100	100	100	100	100	100	100
38	97.5	100	100	100	100	100	100
49	100	100	100	100	100	100	100
96	100	100	100	100	100	100	100

SNP: single-nucleotide polymorphism.

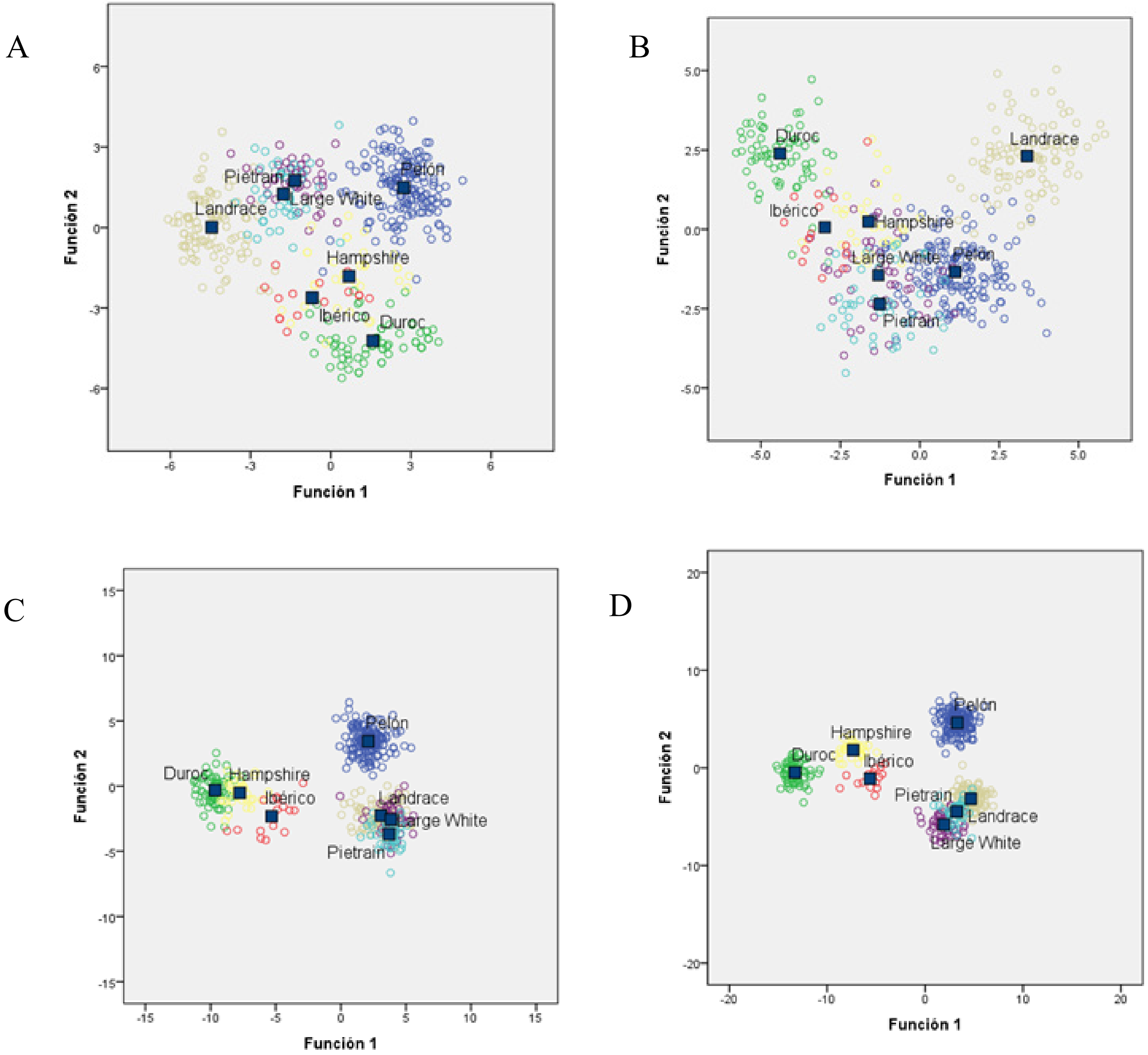

Figure 1. Plots of canonical variables 1 and 2. A, B, C and D, panels with 12, 38, 49 and 96 SNPs, respectively.

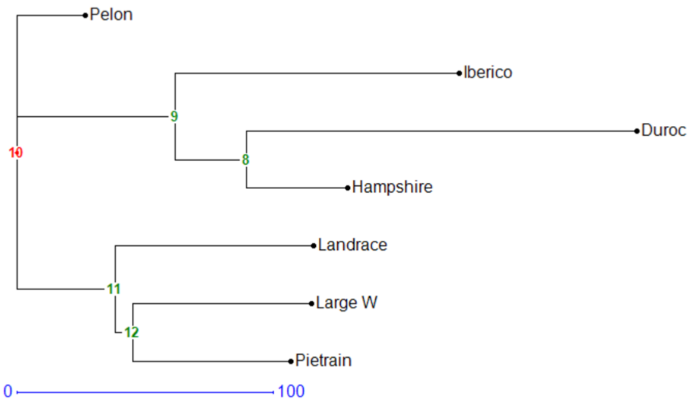

Figure 2. **Dendrogram of the square distances between racial groups with the 96-SNP panel.**

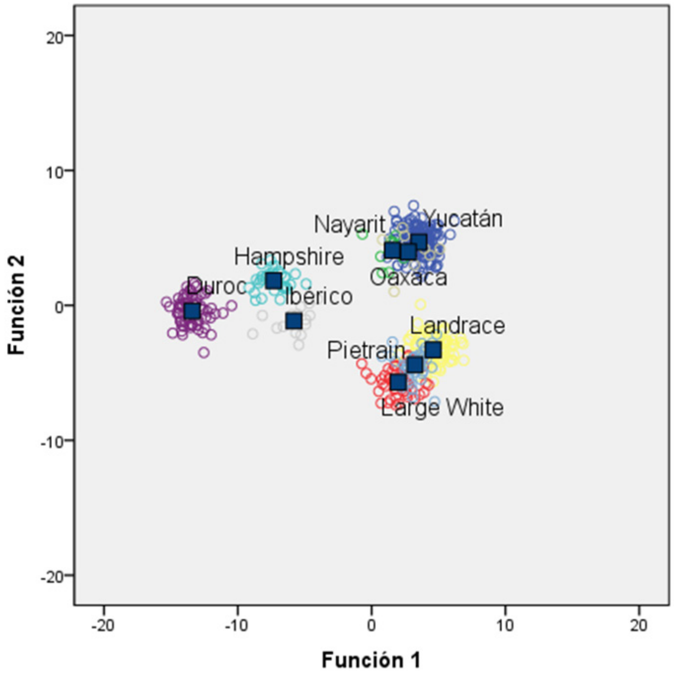

Figure 3. **Clustering of Hairless pig populations using canonical variables 1 and 2 with the 96-SNP panel.**

Despite the differences between the four SNP panel strategies tested, racial authentication is possible with any of these strategies. The greater the number of SNPs in the panel is, the more the canonical correlation of the first two variables in the CDA increases. Moretti *et al*. (2022), with a panel of 20 SNPs and a 60K chip, were able to discriminate Nero Sicilian pigs from other commercial breeds via CDA. Compared with the results obtained with 12 SNPs in this study, the previous study results were similar, as they managed to assign 100 % of the races with the two chips (50K and 60K). In the study published by Moretti *et al.* (2022), the Landrace and Large White pigs were determined to be very genetically close populations, a result similar to that obtained with the 12 SNPs in this study. In the study by Schiavo *et al*. (2020) 96 SNPs were identified via six methodologies; in this study, the panel was reduced to 38 common SNPs using both the 50K and 60K chips. This panel achieved a racial assignment of 100 % in all breeds except Hairless pigs (97.5 %). Similarly, there was also a minimal grouping failure with this panel, with a 99 % allocation when using FST and CDA in commercial breeds, Nero Siciliano, Casertana and Cinta Senese. The results suggest that racial assignment confidence depends on the discrimination methodology (Schiavo *et al*., 2020). When analyzing genotypes with SNP chips and combining the statistical methods delta, FST and ln, Zhao *et al*. (2023) achieved 99 % racial assignment. In another study, Dadousis *et al*. (2022) obtained a racial assignment above 97 % using discriminant PCA. These studies allow us to conclude that chips are an optimal strategy for racial identification, regardless of the statistical method used.

Using the panel of 96 SNPs obtained from 14 British races originally proposed by Wilkinson *et al*. (2012) with the Bayesian partial assignment methodology, a complete racial classification was obtained, except in Hampshire, Landrace, Large White and Pietrain, which had 99 % accuracy. Using the Wilkinson *et al*. (2012) panel reduced to 49 SNPs via CDA, a racial assignment of 100 % for the Hairless pig was achieved. These authors grouped the genetically close Landrace, Large White and Pietrain pigs. Combining the three panels into a fourth panel with 96 SNPs, the racial assignment improved to 100 %, with a higher canonical correlation of the first two variables in the CDA. The Hairless pig populations of the three localities are close, and it is possible to discriminate and achieve adequate racial authentication among them using this strategy. The Hairless pig populations were grouped close to the Iberian pig populations, as has been reported in other studies (Lemus-Flores *et al*., 2023). The SNP bases identified in the four panels tested may be used in future analyses. Muñoz *et al*. (2020) used a 64-SNP panel to authenticate Iberian pigs, separating them from Duroc pigs, and proposed the use of this strategy for the regulation of Iberian meat products. Miao *et al*. (2023) evaluated the methodological proposals of discrimination and concluded that racial identification is feasible and that reliability increases with an increasing number of SNPs. Pasupa *et al*. (2020) reported that the number of SNPs used for racial identification can be reduced, but the best strategy for reliable classification is to combine methodologies. In accordance with that theory, the 96-SNP panel, combining the three previously reported panels, resulted in better racial separation in the Hairless pig in this study.

Conclusions

Using SNP panels identified by 50K or 60K chips, racial authentication of the Hairless pig can be achieved, as it can be discriminated from other breeds. The four strategies evaluated achieved racial separation; the greater the number of SNPs used, the better the identification of the Hairless pig. The 96-SNP panel achieved 100 % racial assignment and a higher canonical correlation of the first two variables in the CDA.

Literature cited

Dadousis, C., Muñoz, M., Óvilo, C., Fabbri, M.C., Araújo, J.P., Bovo, S., Potokar, M.Č., Charneca, R., Crovetti, A., Gallo, M., García-Casco, J.M., Karolyi, D., Kušec, G., Martins, J.M., Mercat, M.J., Pugliese, C., Quintanilla, R., Radović, Č., Razmaite, V., Ribani, A., Riquet, J., Savić, R., Schiavo, G., Škrlep, M., Tinarelli, S., Usai, G., Zimmer, C., Fontanesi, L., & Bozzi, R. (2022). Admixture and breed traceability in European indigenous pig breeds and wild boar using genome-wide SNP data. *Scientific Reports*, *12*, 7346. https://doi.org/10.1038/s41598-022-10698-8.

FAO "Food and agriculture organization". (2022). Domestic animal diversity information system (DAD-IS). http://www.fao.org/dad-is/regionalnational-nodes/efabis/en/.

Lemus-Flores, C., Prado, J. O. B., Bernal, R. V., Correa, J. C. S., & Sansor-Nah, R. (2023). Genetic relationships of the Yucatan black hairless pig with Iberian breeds using single nucleotide polymorfisms. *Brazilian Journal of*

Veterinary Research and Animal Science, *60*, e195697-e195697. https://doi.org/10.11606/issn.1678-4456.bjvras.2023.195697

Miao, J., Chen, Z., Zhang, Z., Wang, Z., Wang, Q., Zhang, Z., & Pan, Y. (2023). A web tool for the global identification of pig breeds. *Genetics Selection Evolution*, *55*, 18. https://doi.org/10.1186/s12711-023-00788-0.

Moretti, R., Criscione, A., Turri, F., Bordonaro, S., Marletta, D., Castiglioni, B., & Chessa, S. (2022). A 20-SNP Panel as a Tool for Genetic Authentication and Traceability of Pig Breeds. *Animals, 12*(11), 1335. https://doi.org/10.3390/ani12111335.

Muñoz, M., García-Casco, J. M., Alves, E., Benítez, R., Barragán, C., Caraballo, C., & Silió, L. (2020). Development of a 64 SNV panel for breed authentication in Iberian pigs and their derived meat products. *Meat Science*. *167*, 108152. https://doi.org/10.1016/j.meatsci.2020.108152.

Óvilo, C., Trakooljul, N., Núñez, Y., Hadlich, F., Murani, E., Ayuso, M., & Muñoz, M. (2022). SNP discovery and association study for growth, fatness and meat quality traits in Iberian crossbred pigs. *Scientific Reports*, *12*,16361. https://doi.org/10.1038/s41598-022-20817-0.

Palma-Granados, P., García-Casco, J. M., Caraballo, C., Vázquez-Ortego, P., Gómez-Carballar, F., Sánchez-Esquiliche, F., & Muñoz, M. (2023). Design of a low-density SNP panel for intramuscular fat content and fatty acid composition of backfat in free-range Iberian pigs. *Journal of Animal Science*, 101, skad079. https://doi.org/10.1093/jas/skad079.

Pasupa, K., Rathasamuth, W., & Tongsima, S. (2020). Discovery of significant porcine SNPs for swine breed identification by a hybrid of information gain, genetic algorithm, and frequency feature selection technique. *BMC Bioinformatics*, *21*, 216. https://doi.org/10.1186/s12859-020-3471-4.

Schiavo, G., Bertolini, F., Galimberti, G., Bovo, S., Dall'Olio, S., Costa, L. N., & Fontanesi, L. (2020). A machine learning approach for the identification of population-informative markers from high-throughput genotyping data: application to several pig breeds. *Animal, 14*(2), 223-232. https://doi.org/10.1017/S1751731119002167.

Wang, Z., Zhang, Z., Chen, Z., Sun, J., Cao, C., Wu, F., Xu, Z., Zhao, W., Sun, H., Guo, L., Zhang, Z., & Pan, Y. (2022). PHARP: A pig haplotype reference panel for genotype imputation. *Scientific Reports*, *12*, 12645. https://doi.org/10.1038/s41598-022-15851-x.

Wilkinson, S., Wiener, P., Archibald, A. L., Law, A., Schnabel, R. D., McKay, S. D., & Ogden, R. (2011). Evaluation of approaches for identifying population informative markers from high density SNP Chips. *BMC Genetics*, *12*, 45. https://doi.org/10.1186/1471-2156-12-45.

Wilkinson, S., Archibald, A. L., Haley, C. S., Megens, H. J., Crooijmans, R. P., Groenen, M. A., & Ogden, R. (2012). Development of a genetic tool for product regulation in the diverse British pig breed market. *BMC Genomics*, 13, 580, https://doi.org/10.1186/1471-2164-13-580.

Yang, B., Cui, L., Perez-Enciso, M., Traspov, A., Crooijmans, R. P., Zinovieva, N., Schook, L., Archibald, A., Gatphayak, K., Knorr, C., Triantafyllidis, A., Alexandri, P., Semiadi, G., Hanotte, O., Dias, D., Dovc, P., Uimari, P. lacolina, L., Scandura, M., Groenen, M., Huang, L. & Megens, H. J. (2017). Genome-wide SNP data unveils the globalization of domesticated pigs. *Genetics Selection Evolution*, *49*, 71. https://doi.org/10.1186/s12711-017-0345-y.

Zhang, C. Y., Wang, Z., Bruce, H. L., Janz, J., Goddard, E., Moore, S., & Plastow, G. S. (2014). Associations between single nucleotide polymorphisms in 33 candidate genes and meat quality traits in commercial pigs. *Animal Genetics*, *45*(4), 508-516. https://doi.org/10.1111/age.12155.

Zhao, C., Wang, D., Teng, J., Yang, C., Zhang, X., Wei, X., & Zhang, Q. (2023). Breed identification using breed-informative SNPs and machine learning based on whole genome sequence data and SNP chip data. *Journal of Animal Science and Biotechnology*, *14*, 85. https://doi.org/10.1186/s40104-023-00880-x.